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• LETTER TO THE EDITOR •

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RoTFL1c of *Rosa multiflora* has a dual-function in suppressing reproductive growth and promoting vegetative growth of *Arabidopsis*

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Dear Editor,

TFL1 homologues play a key role in the flowering habit of roses. To analyze the function of the newly isolated *TFL1* gene of *Rosa multiflora* (Chen et al., 2013), named *RoTFL1c*, we performed overexpression analyses in *Arabidopsis thaliana*. When compared with empty vector control and wild type *Arabidopsis* plants, *RoTFL1c* overexpressing transgenic plants exhibited strong phenotypes such as a clustered habit, an increased number of rosette leaves, late flowering or failure to flower. These phenotypes were basically consistent with, but more resilient than, those observed with overexpression of *TFL1* or its Rosaceae homologous genes in *Arabidopsis*. We concluded that the rose *RoTFL1c* gene not only plays important roles in delaying and/or suppressing flowering, but also in promoting vegetative growth of the plant.

The cDNA of *RoTFL1c* was cloned from *R. multiflora* "Albo-plena" by RT-PCR with specific primers (Table S1 and Figure S1 in Supporting Information). We compared the amino acid sequence of *RoTFL1c* with those of *TFL1* homologues. The AA sequence identities between RoTFL1c and other TFL1 homologues varied depending on their taxonomic relations. The AA identities were 76% with TFL1 of *A. thaliana* and 87% or more when compared with other plants in Rosaceae. The highest identity in the rose family was 99% with *RoKSN* of *Rosa chinensis* var. *spontanea* (Figure S2 in Supporting Information). This is consistent with the high identities of homologous sequences of *TFL1* in Rosaceae (Iwata et al., 2012).

To overexpress *RoTFL1c* in *Arabidopsis*, the ORF fragment of *RoTFL1c* was amplified by PCR with primers (Table S1 in Supporting Information), double-digested with *Xba* I and *Sac* I, and cloned into the plant expression vector pBI121 under the direction of the CaMV 35S promoter (*RoTFL1c*-OE) (Figure S3 in Supporting Information). The pBI121-RoTFL1c (*RoTFL1c*-OE) and pBI121 plasmids were separately introduced into *Agrobacterium tumefaciens* LBA4404 by electroporation. *Arabidopsis* was transformed using the floral-dip method. The resulting transgenic seeds of the first generation (T_0) were screened on Murashige & Skoog (MS) medium containing 80 mg L^{-1} of kanamycin. In three replicate screenings, 11, 19 and 12 kanamycin-resistant seedlings were obtained. These kanamycin-resistant plants were checked for the presence of the *RTFLc* sequence by PCR and 9, 17 and 13 plants were positive (Figure 1A). In comparison with wild type and pBI121 vector-transformed plants, the phenotypes of *RoTFL1c*-OE plants could be categorized into four classes. Class I plants showed a cluster habit and Class II plants had a significant

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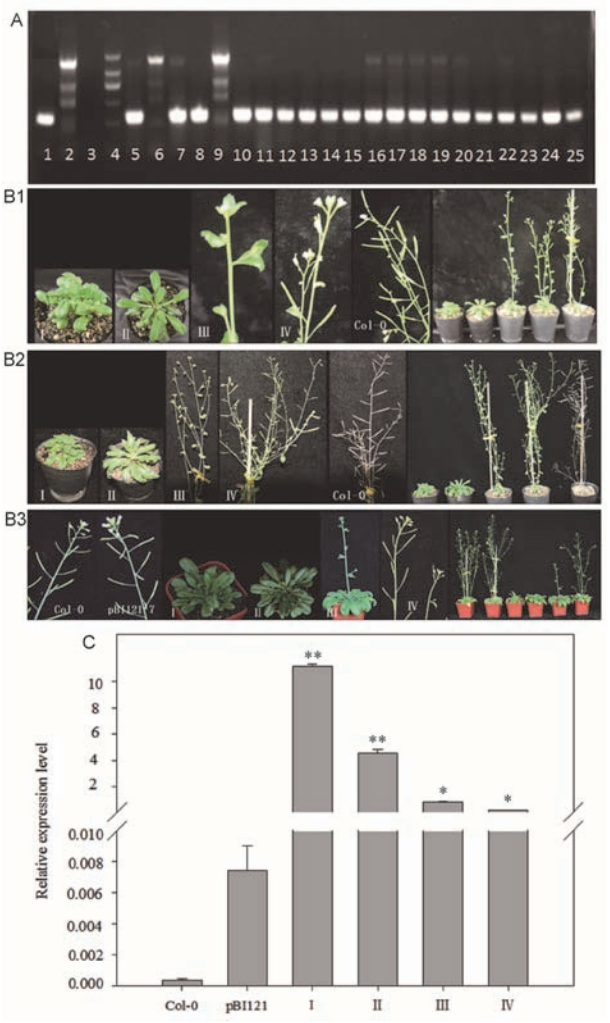

Figure 1 (Color online) Molecular detection and phenotype analysis of *RoTFL1c*-OE transgenic *Arabidopsis thaliana.* A, Molecular detection of transgenic plants. From left to right: 1, positive control; 2, wild type; 3, blank control; 4, DNA ladder marker II; 5–25, transgenic plants. B1, B2, and B3 indicate the different batches of *RoTFL1c*-OE transgenic plants that either showed a delay in blossoming or a failure to blossom. Classes I, II, III and IV indicate different types of phenotypes observed in the *RoTFL1c*-OE transgenic plants. Class I had clustered branches, Class II had a significant number of rosette leaves, Class III had no blossoms, and Class IV showed delayed flowering. Col-0 is the wild type and PBI121 is the *A. thaliana* plant transformed with the pBI121 empty vector. C, A comparison of the expression levels in *RoTFL1c*-OE transgenic plants with different phenotypes. Error bars indicate SE (n=3). * and ** indicate significant difference at $P \leqslant 0.05$ and $P \leqslant 0.01$, respectively, in an SPSS t-test.

increase in the number of rosettes. Neither class bolted after a 4-month growth period. The flower buds of Class III plants failed to open at both the top of the stem and its lateral branches, or aborted after bloom, although there was an increased number of branches. Class IV plants, although they had normal flowers, showed delayed flowering (Figure 1B). All four classes showed similar results in all three batches (Table S2 in Supporting Information).

The expression level of *RoTFL1c* in transgenic plants and wild type *Arabidopsis* was analyzed using real-time PCR. The results showed that the expression level of *RoTFL1c* in Class III, which did not flower, was clearly higher than that in Class IV (Figure 1C), while the expression levels in Classes II and I were even higher than those in Classes III and IV. The results showed that the expression level of *RoTFL1c* was negatively associated with flowering.

There were four classes of phenotypes in the transgenic plants overexpressing *RoTFL1c*. These classes were closely related with the expression level of *RoTFL1c*. With increased levels of *RoTFL1c* expression from Class IV to Class I, the reproductive growth of transgenic plants was increasingly suppressed or vegetative growth was promoted. Such strong variations as a cluster habit (Class I) or an increase in rosettes without shoots (Class II) have not been seen before in transgenic *A. thaliana* carrying *TFL1* homologues. The phenotypes of Class III were similar to those of *A. thaliana* overexpressing *TFL1* homologues from *Cornus officinalis* and *Saccharum officinarum*. The phenotypes of Class IV were similar to those found in *A. thaliana* overexpressing *TFL1* homologues from *Prunus mume*, *Prunus persica* and *Prunus serotina*. They were also similar to those in *tfl1-1* mutant *A. thaliana* overexpressing *TFL1* homologues from *Malus domestica* and *R. chinensis* var. *spontanea* in (Kotoda and Wada, 2005; Randoux et al., 2014), which restored the mutation.

The function of *TFL1* was found to be different between annuals and perennials. For example, mutation of *FvTFL1* caused recurrent flowering in *Fragaria vesca*. The loss of function of *TFL1* homologues from apple and pear by antisense technology resulted in recurrent flowering in transgenic plants. The reason for the nonexpression of *RoKSN* in continuously flowering roses was found to be a retrotransposon insertion (Wang et al., 2012). However, overexpression of *RoKSN* in continuously flowering roses resulted in no flowering at all. Although the function of *TFL1* is universal in Rosaceae as a flowering inhibitor, the corresponding phenotypes (including continuous flowering, recurrent blooming, flowering once, and no flowering types), may be caused by different expression levels of *TFL1* homologues or by other factors associated with the flowering habits of roses.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Electrophoresis analysis of the RoTFL1c ORF fragment.

Figure S2 Sequence alignment (A) and phylogenic tree (B) of TFL1 protein homologues in Rosaceae and Arabidopsis thaliana.

Figure S3 Sketch map of the plant overexpression vector pBI121-RoTFL1c.

Table S1 Primers used in this study

Table S2 Statistics of RoTFL1c-OE transgenic Arabidopsis thaliana with various phenotypes

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